Acetaldehyde

UV-method

for the determination of acetaldehyde in foodstuffs and other materials

Cat. No. 10 668 613 035

Test-Combination for 3 × 11 determinations

BOEHRINGER MANNHEIM / R-BIOPHARM
Enzymatic BioAnalysis / Food Analysis

For use in foodstuff hygiene only. **Store at 2-8°C**

For recommendations for methods and standardized procedures see references (2)

Principle (Ref. 1)

Acetaldehyde is quantitatively oxidized to acetic acid in the presence of aldehyde dehydrogenase (AI-DH) and nicotinamide-adenine dinucleotide (NAD).

$$\text{Acetaldehyde} + NAD^+ + H_2O \xrightarrow{\text{AI-DH}} \text{acetic acid} + NADH + H^+$$

The amount of NADH formed is stoichiometric to the amount of acetaldehyde. NADH is determined by means of its light absorbance at 334, 340 or 365 nm.

The Test-Combination contains

1. Bottle 1 with approx. 100 ml solution, consisting of:
   potassium diphosphate buffer, pH approx. 9.0
2. Bottle 2 with approx. 30 tablets; each tablet contains:
   NAD, approx. 0.8 mg
3. Three bottles 3 with lyophilizate aldehyde dehydrogenase, approx. 4 U, each

Preparation of solutions

1. Use solution of bottle 1 undiluted.
2. Dissolve **one** tablet of bottle 2 with **3 ml** solution of bottle 1 in a beaker or in a centrifuge tube for each assay (blank and samples) depending on the number of determinations. Use forceps for taking the tablets out of bottle 2. For acceleration of the solubilisation crush the tablet with a glass rod; this results in reaction mixture 2.
3. Dissolve contents of one bottle 3 with 0.6 ml redist. water.

Stability of reagents

Solution 1 is stable at 2-8°C (see pack label).
Bring solution 1 to 20-25°C before use.
The contents of bottle 2 are stable at 2-8°C (see pack label).
Reaction mixture 2 is stable for one week at 2-8°C.
Bring reaction mixture 2 to 20-25°C before use.
The contents of the bottles 3 are stable at 2-8°C (see pack label).
Solution 3 is stable at 2-8°C for one week.

Procedure

Wavelength[1]: 340 nm, Hg 365 nm or Hg 334 nm
Glass cuvette[2]: 1.00 cm light path
Temperature: 20-25°C
Final volume: 3.250 ml
Read against air (without a cuvette in the light path) or against water or against blank[3].
Sample solution: 0.5-20 µg acetaldehyde/assay[4] (in 0.200-0.500 ml sample volume).

Pipette into cuvettes	Blank	Sample
reaction mixture 2	3.000 ml	3.000 ml
sample solution*	-	0.200 ml
redist. water	0.200 ml	-
Mix**, after approx. 3 min read absorbances of the solutions (A_1). Start reaction by addition of:		
solution 3	0.050 ml	0.050 ml
Mix**, after completion of the reaction (approx. 3-5 min) read absorbances of the solutions immediately one after the other (A_2)		

It is absolutely necessary to stopper the cuvettes during measurement, e.g. with Parafilm.

Determine the absorbance differences (A_2-A_1) for both, blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample:

$$\Delta A = (A_2\text{-}A_1)_{sample} - (A_2\text{-}A_1)_{blank}$$

The absorbance differences measured should as a rule be at least 0.100 absorbance units to achieve sufficiently precise results (see "Instructions for performance of assay" and "Sensitivity and detection limit", pt.4).

Calculation

According to the general equation for calculating the concentration:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

V = final volume [ml]
v = sample volume [ml]
MW = molecular weight of the substance to be assayed [g/mol]
d = light path [cm]
ε = extinction coefficient of NADH at:
340 nm = 6.3 [l × mmol^{-1} × cm^{-1}]
Hg 365 nm = 3.4 [l × mmol^{-1} × cm^{-1}]
Hg 334 nm = 6.18 [l × mmol^{-1} × cm^{-1}]

It follows for acetaldehyde:

$$c = \frac{3.250 \times 44.05}{\varepsilon \times 1.00 \times 0.200 \times 1000} \times \Delta A = \frac{0.7158}{\varepsilon} \times \Delta A \text{ [g acetaldehyde/l sample solution]}$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor F.

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the result is to be calculated from the amount weighed:

$$\text{Content}_{acetaldehyde} = \frac{c_{acetaldehyde} \text{ [g/l sample solution]}}{\text{weight}_{sample} \text{ in g/l sample solution}} \times 100 \text{ [g/100 g]}$$

1. Instructions for performance of assay

The amount of acetaldehyde in the assay should range between 1 µg and 20 µg (measurement at 365 nm) or 0.5 µg and 10 µg (measurement at 340, 334 nm) resp. The sample solution must therefore be diluted sufficiently to yield an acetaldehyde concentration between 0.02 and 0.10 g/l or 0.01 and 0.05 g/l, respectively.

Dilution table

Estimated amount of acetaldehyde per liter		Dilution with water	Dilution factor F
measurement at			
340 or 334 nm	365 nm		
< 0.05 g	< 0.10 g	–	1
0.05-0.5 g	0.10-1.0 g	1 + 9	10
0.5-5.0 g	1.0-10 g	1 + 99	100
> 5.0 g	> 10 g	1 + 999	1000

* Rinse the enzyme pipette or the tip of the piston pipette with sample solution before dispensing the sample solution.

** For example, with a plastic spatula or by gentle swirling after closing the cuvette with Parafilm (trademark of the American Can Company, Greenwich, Ct.,USA).

1 The absorption maximum of NADH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; when spectralline photometers equipped with a mercury vapour lamp are used, measurements are taken at a wavelength of 365 nm **or** 334 nm.
2 If desired disposable cuvettes may be used instead of glass cuvettes.
3 For example when using a double-beam spectrophotometer.
4 See instructions for performance of assay.

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Because of the volatility of acetaldehyde, the dilution of samples should be carried out as follows:

Fill the volumetric flask approx. to the half with water and pipette the sample with an enzyme test pipette or a piston type pipette under the surface of the water. Fill up to the mark with water and mix.

If the measured absorbance difference (ΔA) is too low (e.g. < 0.100), the sample solution should be prepared again (weigh out more sample or dilute less strongly) *or* the sample volume to be pipetted into the cuvette can be increased up to 0.500 ml. The volume of solution 1 or reaction mixture 2, respectively, remains the same (3.000 ml).

The volume of water pipetted into the blank assay must then be increased so as to obtain the same final volume in the assays for sample and blank. The new sample volume (v) and the new final volume (V) must be taken into account in the calculation.

2. Technical information

2.1 Acetaldehyde is extremely volatile (boiling point approx. 21°C); therefore, all containers with samples, sample and assay control solutions have always to be closed tightly.

2.2 Because of the high volatility of acetaldehyde, it is always necessary to pipette acetaldehyde containing solutions under the surface of water or buffer solutions e.g. when preparing sample or assay control solutions esp. when diluting these materials, or when dispensing these solutions into the cuvette.

2.3 Acetaldehyde is easily to be oxidized (in the presence of oxygen from air) forming acetic acid; therefore, samples have to be analyzed as soon as possible after sampling. Assay control solutions are only stable for some time, too.

2.4 Acetaldehyde polymerizes after some storage time. The polymerized acetaldehyde does not react in the enzymatic assay system. Therefore, acetaldehyde must be destilled freshly immediately before preparing the assay control solution.

As an alternative acetaldehyde ammonia(1-amino ethanol) may be used as an assay control material.

2.5 Acetaldehyde has a strong biting smell; acetaldehyde irritates mucous membranes and has narcotic properties. Therefore, acetaldehyde should be stored at −15 to −25°C before an assay control solution is produced. It is also recommended to pipette acetaldehyde with a glass pipette, stored also at −15 to −25°C, under the surface of water filled into the volumetric flask. (Close the volumetric flask always before weighing; see pt. 2.1.)

3. Specificity

AI-DH also converts, though with much lower velocity, other aldehydes like propionaldehyde, glycolaldehyde and benzaldehyde. In the presence of these aldehydes acetaldehyde can be measured by extrapolation of A_2 to the time of the addition of the solution 3 (AI-DH). Under the above-mentioned assay conditions the oxidation of formaldehyde, crotonaldehyde, glyceraldehyde is so unimportant that its influence on the determination of acetaldehyde can be excluded, even on a high excess.

In the analysis of commercial freshly distilled acetaldehyde (see pt. 2.4) results of approx. 100% have to be expected. (A recovery of less than 100% does not mean an inclompete conversion in the enzymatic reaction, it rather indicates the loss of acetaldehyde during the handling of the sample and preparing the sample solution, as well as when pipetting the diluted acetaldehyde solution into the assay system.)

Note:
With the determination of acetaldehyde in samples, that also contain sulfite "total acetaldehyde" is measured, that means the sum of free and on sulfite bound acetaldehyde.

4. Sensitivity and detection limit (Ref. 1.2)

The smallest differentiating absorbance for the procedure is 0.005 absorbance units. This corresponds to a maximum sample volume v = 0.500 ml, an assay volume V = 3.550 ml and measurement at 340 nm of an acetaldehyde concentration of approx. 0.25 mg/l sample solution (if v = 0.100 ml and V = 3.150 ml, this corresponds to approx. 1 mg/l sample solution).

The detection limit of 1 mg/l is derived from the absorbance difference of 0.020 (as measured at 340 nm), a maximum sample volume v = 0.500 ml and an assay volume V = 3.550 ml.

5. Linearity

Linearity of the determination exists from approx. 0.5 µg acetaldehyde/assay (1 mg acetaldehyde/l sample solution; sample volume v = 0.500 ml; assay volume V = 3.550 ml) to 20 µg acetaldehyde/assay (0.2 g acetaldehyde/l sample solution; sample volume v = 0.100 ml; assay volume V = 3.150 ml).

6. Precision

In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of v = 0.200 ml, an assay volume of V = 3.250 ml and measurement at 340 nm, this corresponds to an acetaldehyde concentration of approx. 0.5 - 1 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of approx. 0.005-0.01 g/100 g can be expected).

The following data have been published in the literature:

CV = 2.2 %			(Ref. 1.1)
x = 4 µg/assay	CV = 3.7 %	n = 16	
x = 20 µg/assay	CV = 0.77 %	n = 16	(Ref. 1.2)

7. Interference/sources of error

Components of animal materials do not interfere in the determination of acetaldehyde. (Poly-) phenols from plants reduce the speed of reaction.

Alcohols do not interfere with the assay even on a high excess. Also reducing substances like ascorbic acid, sulfur dioxide (up to 50 µg SO_2/assay) do not affect the acetaldehyde assay.

8. Recognizing interference during the assay procedure

8.1 If the conversion of acetaldehyde has been completed according to the time given under "Procedure", it can be concluded in general that no interference has occurred.

8.2 On completion of the reaction, the determination can be restarted by adding acetaldehyde (resp. of acetaldehyde ammonia = 1-amino-ethanol; qualitative or quantitative): if the absorbance is altered subsequent to the addition of the standard material, this is also an indication that no interference has occurred.

8.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carrying out a double determination using two different sample volumes (e.g. 0.100 ml and 0.200 ml): the measured differences in absorbance should be proportional to the sample volumes used.

When analyzing solid samples, it is recommended that different quantities (e.g. 1 g and 2 g) be weighed into 100 ml volumetric flasks. The absorbance differences measured and the weights of sample used should be proportional for identical sample volumes.

8.4 Possible interference caused by substances contained in the sample can be recognized by using an internal standard as a control: in addition to the sample, blank and standard determinations, a further determination should be carried out with sample **and** assay control solution in the same assay. The recovery can then be calculated from the absorbance differences measured.

8.5 Possible losses during the determination can be recognized by carrying out recovery tests: the sample should be prepared and analyzed with and without added standard material. The additive should be recovered quantitatively within the error range of the method.

9. Reagent hazard

The reagents used in the determination of acetaldehyde are not hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulation 67/548/EEC and subsequent alteration, supplementation and adaptation guidelines. However, the general safety measures that apply to all chemical substances should be adhered to.

After use, the reagents can be disposed of with laboratory waste, but local regulations must always be observed. Packaging material can be disposed of in waste destined for recycling.

10. General information on sample preparation

In carrying out the assay:
Use **clear, colorless and practically neutral liquid samples** directly, or after dilution according to the dilution table (in order to avoid a loss of acetaldehyde, it is highly recommended to pipette the sample always under the surface of the diluent), and of a volume up to 0.500 ml;
Filter **turbid solutions** (a loss of small amounts of acetaldehyde is possible);
Degas **samples containing carbon dioxide** (e.g. by filtration or in order to avoid a loss of acetaldehyde, by the addition of solid KOH or NaOH in order to bind CO_2 as bicarbonate);
Adjust **acid samples** to pH 8-9 by adding sodium or potassium hydroxide solution;
Adjust **acid and weakly colored samples** to pH 8-9 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min;
Measure **"colored" samples** (if necessary adjusted to pH 8-9) against a sample blank (= buffer or redist. water + sample), adjust the photometer to 0.000 with the blank in the beam;
Treat **"strongly colored" samples** that are used undiluted or with a higher sample volume with polyamide or polyvinylpolypyrrolidone (PVPP), or with activated charcoal, e.g. 2 g/100 ml;
Crush or homogenize **solid or semi-solid samples,** extract with water or dissolve in water and filter if necessary; resp. remove turbidities or dyestuffs by Carrez clarification;
Deproteinize **samples containing protein** with perchloric acid; alternatively clarify with Carrez reagents;
Extract **samples containing fat** with hot water in a volumetric flask with a condenser (extraction temperature should be above the melting point of the fat involved). Cool to allow the fat to separate, rinse the condenser with redist. water, make up to the mark, place the volumetric flask in an ice bath for 15 min and filter; alternatively clarify with Carrez-solutions after the extraction with hot water.

Carrez clarification:

Pipette the liquid sample into a 100 ml volumetric flask containing approx. 60 ml redist. water, or weigh sufficient quantity of the sample into a 100 ml volumetric flask and add approx. 60 ml redist. water. Subsequently, carefully add 5 ml Carrez-I-solution (potassium hexacyanoferrate(II) (ferrocyanide), 85 mM = 3.60 g $K_4[Fe(CN)_6] \times 3\ H_2O$/100 ml) and 5 ml Carrez-II-solution (zinc sulfate, 250 mM = 7.20 g $ZnSO_4 \times 7\ H_2O$/100 ml). Adjust to pH 7.5-8.5 with sodium hydroxide (0.1 M; e.g. 10 ml). Mix after each addition. Fill the volumetric flask to the mark, mix and filter.

11. Application examples

Determination of acetaldehyde in fruit juices
Filter turbid juices, adjust pH of colored juices to approx. 8.0 and incubate for approx. 15 min; measure against a sample blank.

Decolorize strongly colored juices with activated charcoal, e.g. 2 g/100 ml; if necessary dilute according to the dilution table, or use a sample volume up to 0.500 ml.

Determination of acetaldehyde in wine (Ref. 3.1, 3.2)
Adjust white wine to pH 8.0 with 2 M sodium hydroxide. Immediately use 0.100-0.500 ml for the assay.

Note:
With the determination of acetaldehyde in samples, that also contain sulfite "total acetaldehyde" is measured, that means the sum of free and on sulfite bound acetaldehyde.

Determination of acetaldehyde in red wine (Ref. 3.1, 3.2)
Decolorize red wine, if necessary, before using for the assay, as follows: Mix 10 ml red wine and 0.2 g activated charcoal; stir for approx. 30 s and filter rapidely. Use the clear and mostly colorless solution without further dilution. Use 0.200 for the assay. (Generally, neutralisation is not necessary after decolorization.)

Note:
With the determination of acetaldehyde in samples, that also contain sulfite "total acetaldehyde" is measured, that means the sum of free and on sulfite bound acetaldehyde.

Determination of acetaldehyde in liquors
Use brandies undiluted with v = 0.200 ml (0.500 ml, if necessary) directly for the assay.

Determination of acetaldehyde in beer (Ref. 3.3) and champagne
For removal of CO_2 shake samples in a beaker for 30 s, or add solid KOH or NaOH up to a pH 9 (in the analysis of colored samples) in order to bind CO_2 as bicarbonate; if necessary dilute according to the dilution table, or use a sample volume up to 0.500 ml.

Determination of acetaldehyde in yogurt and fruit-yogurt
Accurately weigh approx. 40 g yogurt (or fruit-yogurt, respectively) into a beaker, add 4 ml solution of citric acid (20%, w/v), stir slightly, transfer the solution into a 50 ml volumetric flask with water and subsequently fill up to the mark with water. Mix and filter through a fluted filter paper, centrifuge, if necessary. Discard the first few ml; use the usually clear solution with v = 0.200 ml for the assay.

Determination of acetaldehyde in fruit and vegetable products, in bread and in low-fat dairy products
Accurately weigh the homogenized sample into a 100 ml volumetric flask, extract with water (shake the closed flask vigorously). Fill the volumetric flask to the mark, mix and filter. If the filtrate is not clear enough, clarify with Carrez-solutions.
If necessary dilute according to the dilution table, or use a sample volume up to 0.500 ml.

Determination of acetaldehyde in coffee, cocoa and fat-containing milk products (e.g. butter)
Accurately weigh the homogenized sample into a 100 ml volumetric flask, extract with warm water at a temperature above the melting point of fat using a condenser. Rinse the condenser with water, adjust to 20 to 25°C and fill the volumetric flask up to the mark. Put the volumetric flask into an ice-bath or into a refrigerator for approx. 15 min and filter the cold material.
If necessary dilute according to the dilution table, or use a sample volume up to 0.500 ml.

12. Further applications

This method may also be used in research when analyzing biological samples.

For details of sampling, treatment and stability of the sample see Bernt, E. & Bergmeyer, H. U. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 2nd ed., vol. 3, p. 1506, Verlag Chemie, Weinheim/Academic Press, Inc., New York and London, as well as Ref. 1.

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Acetaldehyde assay control solution

Besides acetaldehyde (s. pt. 2.4), acetaldehyde ammonia (= 1-amino ethanol), e.g. from Fluka/Buchs (Switzerland), Cat. No. 00090, is also suited to be used as an assay control material for checking the assay procedure.

Note

Acetaldehyde ammonia is irritating for the skin and flammable. When handling the material, the general safety measures that apply to all chemical substances should be adhered to. In the case of contact with skin wash thoroughly with water, in the case of contact with the eyes rinse carefully with water for 15 min. If necessary contact ophthalmologist.

Assay control solution

Dissolve approx. 80 mg of the assay control material (which corresponds to approx. 50 mg acetaldehyde) in 1 l of redist. water. Prepare freshly before use.

Application:

1. Addition of acetaldehyde assay control solution to the assay mixture:
Instead of sample solution the assay control solution is used for the assay. (The measurement of the assay control solution is not necessary for calculating the results.)

2. Restart of the reaction, quantitatively:
After completion of the reaction with sample solution and measuring of A_2, add 0.100 ml assay control solution to the assay mixture. Read absorbance A_3 after the end of the reaction (approx. 5 min). Calculate the concentration from the difference of (A_3-A_2) according to the general equation for calculating the concentration. The altered total volume must be taken into account. Because of the dilution of the assay mixture by addition of the assay control solution, the result differs insignificantly from the expected value.

3. Internal standard:
The assay control solution can be used as an internal standard in order to check the determination for correct performance (gross errors) and to see whether the sample solution is free from interfering substances:

Pipette into cuvettes	Blank	Sample	Standard	Sample + Standard
reaction mixture 2	3.000 ml	3.000 ml	3.000 ml	3.000 ml
redist. water	0.200 ml	–	–	–
sample solution	–	0.200 ml	–	0.100 ml
assay control sln.	–	–	0.200 ml	0.100 ml
mix, and read absorbances of the solutions (A_1) after approx. 3 min. Continue as described in the pipetting scheme under "Procedure". Follow the instructions given under "Instructions for performance of assay" and the footnotes.				

The recovery of the standard is calculated according to the following formula:

$$\text{recovery} = \frac{2 \times \Delta A_{\text{sample + standard}} - \Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 100\ [\%]$$

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